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POSSIBLE DOMESTICATION OF EDIBLE WILD MUSHROOM *AGARICUS SILVATICUS* G. J. KEIZER IN RIVERS STATE, NIGERIA

Orikoha, E. C. and Dimkpa, S. O. N.
Department of Crop/Soil Science, Rivers State University, Port Harcourt, P.M.B 5080, Rivers State, Nigeria

ABSTRACT: *The experiment on the artificial cultivation of Agaricus silvaticus was conducted in crop protection laboratory at the Rivers State University. Wild edible mushroom fruiting bodies used were collected from three different sites (site one, site two, site three) in Ogwe, Ukwa West Local Government Area, Abia State. Mycelium (basidia) production was significantly achieved and cultivated in a modified ancient Indian traditional method, site two (66.0) significantly had the highest mycelium production compared to site three (49.0) and site one (48.0). The mycelium from 3 sites was used to inoculate the spent grain to achieved spawn production. Site two recorded the highest spawn production (56.0) followed by site one (52.0) and least at site three (39.0). Germination of Agaricus silvaticus pin-heads (primordia) were achieved by inoculating the spawn from the three sites into decomposed palm bunch (DPB) and decomposed saw dust (DSD) for fruiting bodies determination. The experimental results therefore shows significant difference on the relationship between two substrates decomposed palm bunch (DPB) in site one having the highest (53.7) followed by site two (46.3) and site three (41.3) and no growth in decomposed saw dust (DSD). It is note-worthy that this experiment successfully achieved mycelium production, spawn production and cropping of Agaricus silvaticus. This is a major scientific breakthrough and first trial for the cultivation of Agaricus silvaticus in Rivers State and Nigeria at large. Thus, the knowledge will contribute to the commercial production of Agaricus mushroom which is a dietary delicacy in most African Countries though completely dependent on seasonal growth from the wild.*

KEY WORDS: *Agaricus silvaticus*, mycelium, spawn and pin-head germination

INTRODUCTION

Agaricus silvaticus (or *Agaricus sylvaticus*) is a saprophytic fungus belonging to the family *Agaricaceae*. It is also known as Scaly Wood Mushroom, Blushing Wood Mushroom or Pinewood Mushroom (Garnweidner, 1994; O'Reilly, 2016). It grows on soil humus, decaying litter on forest floors, wood logs and manure piles. It is also found in groups in coniferous forests from early summer, or September through to November in Europe, North America, North Africa and Nigeria (Miller *et al.*, 2006; Roger, 2010). *Agaricus* species are the leading mushroom crops worldwide and account for 40% mushroom production. Natural or synthetic substrates may be used in the production systems for domesticated varieties which vary by type of mushroom, including indoor and outdoor systems (Chang and Miles, 2004).

Agaricus is a secondary decomposer i.e. bacteria and other fungi have to break down raw materials before *Agaricus* can grow. The compost must not contain foods upon which other competitor fungi may grow. During composting, nutrients favored by competitor microbes progressively decrease while nutrients selective for the mushroom mycelium accumulate (Samp, 2007). Edible mushrooms are fungi and not vegetables and are either harvested wild or cultivated (O'Reilly, 2016). Mushroom is the fleshy, spore-bearing fruiting body which composed of a stipe (stem), a pileus (cap), and lamellae (gills), other species additionally have a volva (cup), that is *Volvariella volvacea* and annulus (ring) (Hawksworth, 2001).

Structures of *Agaricus* are vegetative mycelium (living inside the soil) and Fruiting body or basidiocarp (present above the soil and edible in young stage). Mushrooms form from a small structure called a primordium which grows on some type of substrate. The primordium enlarges into an egg shaped structure composed of hyphae called a "button" and Mycelium (the universal veil) which initially surrounds the button. As the button grows, the veil breaks and the remnants of the veil on mature mushrooms often appear as warts or may be found hanging from the cap (Khandelwal, 2014). *Agaricus spp*. reproduces vegetatively (through perennating mycelium), asexually (through the production of chlamydospore and oidia) and sexually (through plasmogamy, karyogamy and meiosis) (Khandelwal, 2014). The greyish-brown cap is hemispherical when young, but later flattens out up to 10 cm in diameter and covered with broad scales. The gills are grey when young, and become much darker with age. The spores are chocolate brown. The stem is brownish, often with a hanging ring and a small bulb at the base.

The key factors used in identifying mushroom include color, structure and the environment where it was picked from. A key is usually provided to simplify identification in most reference texts (Arora, 1986; Carluccioi, 2003). Macro- morphological identification based on habitat, shape of cap, cap size, colour (spore print), margin pattern and surface pattern (Osemwegie *et al.,* 2006; Ukoima *et al.*, 2009).The flesh is white with mild taste turning reddish when cut. The young fruit bodies are well suited for consumption. Edible mushroom is a macro-fungus with a distinctive fruiting body which can be either epigeous or hypogeous and large to be seen with the naked eye and to be picked by hand (Chang and Miles, 2004; O'Reilly, 2016).

Nutritionally, edible mushrooms consist of protein (25-50%), fat (2.5%), sugar (17-47%), mycocellulose (7.38%), thiamine (0.12mg/100g sample), riboflavin (0.52mg/100gsample), ascorbic acid (8.60mg/100g sample), nicotinic acid (5.85mg/100g sample), pantothenic acid (2.3mg/100 sample) and are a good source of the B vitamins and niacin (Wang *et al.*, 2003 and 2004). Aletor (1990) and Okwulehie *et al.*, (2017), emphasized that mushrooms are rich in protein, minerals and vitamins. The protein content of mushrooms has been reported to be twice that of vegetables and four times that of oranges (Bano *et al*., 1993), significantly higher than those of wheat (Aletor, 1990), and of high nutritional quality comparing favourably with meat, egg, and milk (Thatoi and Singdevsachan,

2014). Five medium raw mushrooms have only 20 calories, no fat, and provide 3 grams of protein, 3 grams of carbohydrate, and 1 gram of dietary fiber. They are often marketed as a "meat replacer" due to their protein content and fleshy texture. The edible wild *Agaricus* have mostly strong and delicious flavors ranging from a stronger version of the white button to some that are even almondy in smell and flavor.

Agaricus mushroom though commercially cultivated in some country of the world but have not been domesticated in Nigeria and its availability depends solely on seasonal growth in the wild. Commercialization of the cultivation and production of *Agaricus* mushroom to contribute in human nutritional needs in Nigeria is highly imperative for its availability as a substitute to the seasonal wild mushroom. This will as well improve food security, create self-employment and alleviate poverty. Thus, this article document the first trial for the possible domestication of *Agaricus silvaticus* in Rivers State using traditional method of mycelium production, spawn production and pin-head germination (fruiting bodies).

MATERIALS AND METHODS

Experimental site

The research was conducted in the crop protection laboratory, Crop/Soil Science Department at the Rivers State University, Nkpolu-Oroworukwo, Port Harcourt, Nigeria.

Collection of Experimental materials

The mushroom samples used for the experiment were collected from two locations in a natural habitat (Obiahia) in Ogwe, Ukwa West Local Government Area, Abia State (Plate A) where the mushroom was found in the wild while soil samples were collected from 3 different locations in Ogwe (Plate B): 2 from Obiahia kindred the mushroom natural habitats and the third a control plot from Obiawom kindred where mushroom was not found. Malt Spent grain for inoculation was collected from Pabod Breweries Limited, Port-Harcourt, Rivers State. Decomposed saw dust were collected from timber market, Eagle lsland sand filled, Port-Harcourt and Decomposed palm bunch were collected from a local oil palm mill in Omagwa, Ikwere Local Government Area, Rivers State.

Plate A: Agaricus collection in the wild

Plate B: Soil Samples collection

Creating Mycelium (Production)

A modified Traditional India method was used for the production of mushroom Mycelia where the harvested wild Agaricus were divided into small sections sandwiched in sterilized cardboard paper and incubated in a container box for seven days in the laboratory under tropical room temperature at 23-25^{o}C.

Spawn Production

The method of spawn production used was Ruffling- in, this was done according to the methods of Wuest and Bengston (1982) where the produced mycelia were broadcast over the surface of the Malt spent grain and ruffled-in as the substrates was being manipulated into a flat surface. The depth of ruffling-in varies from 1 to 3cm. 400ml beaker was earlier used to measure out the malt spent grain and was transferred into a sterilized white horticultural polythene bags. The sterilization was for 15minutes at 121°C. The solid mycelia (basidia) were then inoculated into the polythene bags containing spent grain and were transferred into an incubator conditioned at 22 ± 1°C with a relative humidity of 60%. When the mycelium was fully colonized in the spent grain substrates polythene bags, they were removed after 21days for the production of fruiting bodies.

Mushroom Cultivation

Three kilogram (3kg) of substrates (Top soil (T1), Decomposed palm bunch mixed with one kilogram (1kg) of top soils (T2) and Decomposed saw dust mixed with one kilogram (1kg) of top soils (T3)

were placed in a white horticultural bag (1.75cm × 15cm width). The bags were earlier sterilized for 15minutes at 121^{o}C and allowed to cool for 7 hours. The spawn from various bags were then seeded into bags containing the substrates and watered enough to create a moist and humid environment for pin-head (primordia) and fruiting bodies of mushroom germination for 18-21 days. The bags of the substrates were placed on screen house floor at room temperature (24^{o}C) for observations of mycelium to reach the surface of the casing layer. Later the air temperature is reduced to 16- 18^{o}C and $C0_2$ concentration to 0.08% for mushroom to induced pin-head. Fruiting bodies required were monitored for 18-21 days after planting. The cultures were watered with 50cl of tap water 2-3 times weekly to keep the substrates moist.

Experimental Design

All treatments for the experiment were laid out using a complete randomization design (CRD)
For mycelium and spawn production, there were 3 treatments and replicated five times:
Site one (T1), Site two (T2), and Site three (T3)
For cropping of *Agaricus silvaticus*, 3 treatments were used and replicated four times:
Top soil (T1), Decomposed palm bunch mixed with topsoil (T2) and Decomposed saw dust mixed with topsoil (T3)
Note: The top soil used in cropping of *Agaricus silvaticus* were collected from the experimental sites.

Statistical Technique

The analysis of variance (ANOVA) was used to determine the treatment effects and means were tested using Tukey means method of grouping at 5% level of probability (Minitab, 2010).

RESULTS

The weather condition as illustrated in Table 1 shows that the natural habitat of the mushroom in Ogwe, Ukwa West Local Government Area, Abia State is located at latitude 5^{0}521N and longitude of 7^{0}161E. It covers about 50km^2 with soils derived from coastal plain sands (Benin Formation) and dominated by flat to gentle slopes landscape. Ogwe is situated in humid tropic with mean annual rainfall of about 2500-3000mm and annual temperature range of 26-31^{o}C. The vegetation is tropical rainforest characterized by varieties of stored plant species.

The mycelium growth was completely absent in control sample. Site two was highly significant ($P < 0.05$) in the production of mycelium with a mean value of (66.0) and site three had (49.0) while site one recorded a mean value of 48.0. However, there was no significant different ($P > 0.05$) between mycelium gotten from site one and site three (Table 2 and Plate 1). The spawn growth in Table 3 illustrated the result of reproductive spawn produced by the various sites samples. Control had no growth, Site two was significantly ($P < 0.05$) highest with mean value of 56.0 followed by site one (52.0), while the least was site three (39.0) though no significant different between site one and site two (Plate 2).

The experimental results in Table 4 showed the germination of pin head in the various soil sample sites. *Agaricus silvaticus* showed significant differences ($P < 0.05$) in the various studied sites. Site one was highly significant ($P < 0.05$) with a mean value of 26. 9. However, site two which has the mean value of 23.1 is not significantly different from site three (20.6) (Plate 3).

The result in Table 5 clearly states the possible growth of pin-head in the various sites soils mixed with decomposed palm bunch and saw dust. Pin-head production in decomposed Palm bunch in site one (53.7) was highly significant ($P< 0.05$) from the one produced in site two with a mean value of 46.3 and site three with mean of 41.3.The experiment recorded no growth of *Agaricus* on the various sites soil samples mixed with decomposed saw dust. It was observed that decomposed saw dust showed no growth.

Table 1: Weather Conditions of the Study Area

Cloudy	**Thunder Shower**	**A few- Storms**
24~32ºC	**24~33ºC**	**24~34ºC**
Wind direction	**Wind speed**	**Temperature**
North	0.00km/h	33ºC
North	0.00km/h	33ºC

Table 2: Mycelium production in each of the soil samples

Samples	Mycelium production
Control	0^{c}
Site one	48.0^{b}
Site two	66.0^{a}
Site three	49.0^{b}

*Means that do not share same letter are significantly different (Tukey method at 98% Confidence level)

Key:

Control - Uzopipeline, Site 1 – Obiahia, Site 2 - Obiahia, Site 3 - Obiahia

Table 3: Spawn production in each of the soil samples

Samples	Spawn production
Control	0^{c}
Site one	52.0^{a}
Site two	56.0^{a}
Site three	39.0^{b}

*Means that do not share same letter are significantly different (Tukey method at 98% Confidence level)

Key:
Control - Uzopipeline, Site 1 – Obiahia, Site 2 - Obiahia, Site 3 - Obiahia

Table 4: Pin- head of the various Substrates samples

Sample	Pin head
Site one	26.9^{a}
Site two	23.1^{ab}
Site three	20.6^{b}

*Means that do not share same letter are significantly different (Tukey method at 98% Confidence level)

KEY:
Control - Uzopipeline, Site 1 – Obiahia, Site 2 - Obiahia, Site 3 - Obiahia

Table 5: Relationship between substrates samples mixed with two treatments in Pin-head

Samples	Mean
Site one DPb	53.7^{a}
Site two DPb	46.3^{b}
Site three DPb	41.3^{b}
Site one DSd	0^{c}
Site two DSd	0^{c}
Site three DSd	0^{c}

*Means that do not share same letter are significantly different (Turkey method at 98% Confidence level)

Key:
Control - Uzopipeline, Site 1 – Obiahia, Site 2 - Obiahia, Site 3 – Obiahia, Dpb– Decomposed Palm bunch, Dsd–Decomposed Sawdust

The regression analysis in Figure 1 showed that the relationship between spawn production and mycelium growth is statistically significant ($P < 0.05$) revealing a positive correlation ($r = 0.93$) as mycelium production increases, spawn production also tend to increases in the various studied sites. There was a positive correlation between spawn growth and in mycelium production, site two was highly significant with a mean value of 66.0, followed by site three with a mean of (49.0) but not significant from site one (48.0). Control had no growth. The variation in spawn production was highly significant ($P < 0.05$) in site two with a mean value of 56.0 followed by site one with a mean value of (52.0), while site one recorded the least (39.0). However control observed no growth just like in the mycelium production.

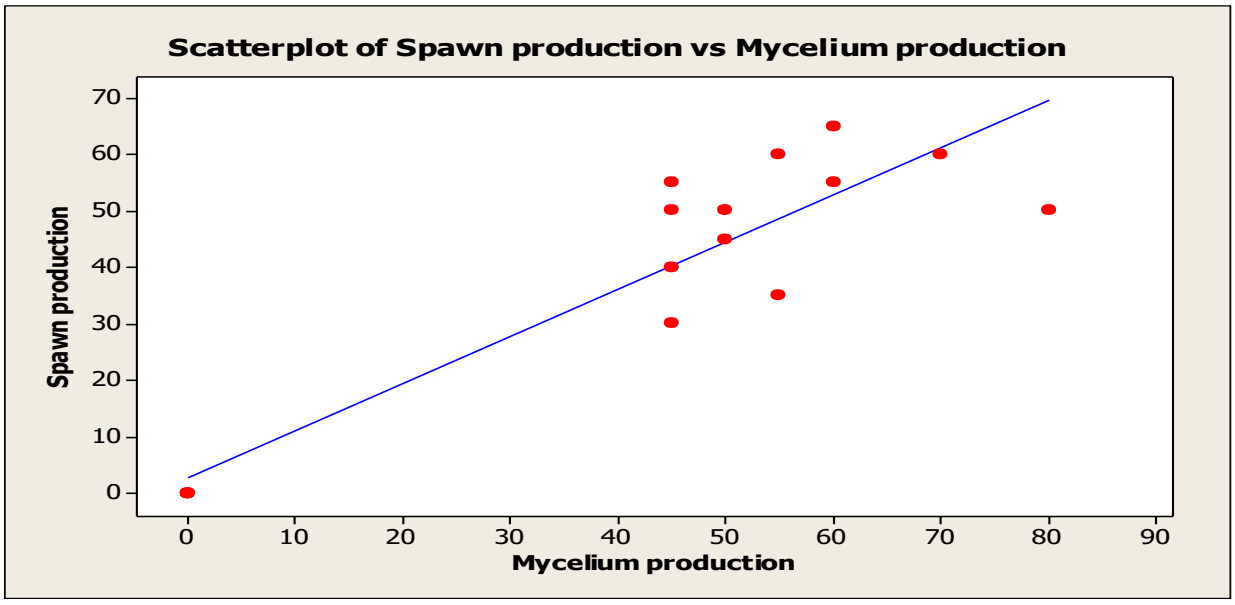

Figure 1: Scatter plot of Spawn production vs Mycelium production

Plate 1a & b: Pictorial view of mycelium site one and site two

Plate 1c: Pictorial view of mycelium site three

Plate 2: Pictorial View of Spawn production (Site One and Site Two)

Plate 3: Pictorial View of Pin-Head (Site One and Site Two)

DISCUSSION

The experimental results and observations most importantly affirmed the fact that *Agaricus* mushroom is highly specific and adapted to its natural wild habitat which is a complete humid tropical rainforest at an annual temperature range of 22-31^{o}C. The vegetation is characterized by varieties of stored and perennial plant species fallowed for about two years. Love *et al.* (1986) reported an ideal temperature for mycelia development as 22-28°C and 15-19°C for fruiting body development. Umar and Van Griensven (2000) further observed that a 4^{0}C increase in temperature induced macroscopic changes in primordium morphogenesis leading to faster fruiting bodies development, early opening of the mushroom veil and increased cap size prior to harvest. These observations agreed with Van-Peer *et al.* (2009), who reported that various mushrooms are known to be sensitive to the climatic conditions particularly temperate and relative humidity.

The artificial mycelium (basidia) production in this research was achieved through traditional method which revealed a good growth of mycelium at the 4th days of incubation. Importantly, the impressive yield in mycelium production from this research attest the success of the traditional methodology applied in this current work for the production of good mycelia growth which is essential and bases for mushroom production. Spawn production of *Agaricus silvaticus* domesticated vegetatively on sterilized spent grain was also successfully achieved in this study through a method of Ruffling-in. This method was reported by Wuest and Bengston (1982) when mechanized spawning equipment was not available. The depth of Ruffing-in varies and the mushroom mycelium grows down through the substrates from wherever the deepest spawn grains happen to end up. In this research, the care of mycelia (spawn) runners that is the phase during which mycelia grows from the spawn and permeate into the substrates was successful as described by Obe and Mshigeni (2013).

However, severe losses of spawn occurred due to contamination of spent grain with green mould, *Aspergillus spp.* and *Penicilium spp.* resulting from interrupted power supply in the course of this research. It was discovered that the contamination may be as a result of malformed mushrooms, which are prone to accelerated postharvest deterioration while infested spores of mycelium and spawning spread the pathogen (Romaine and Schlaghanfer, 1995). The high moisture content of the mushroom and level of deterioration maybe indication that fresh mushroom cannot be kept for a longer time. This is due to the fact that high moisture activity favored microbial growth (Aletor, 1990).

Pin-head of the various substrates sample sites of edible wild mushroom *Agaricus silvaticus* were determined and achieved by inoculating the spawn into decomposed palm bunch and decomposed saw dust bags. Decomposed palm bunch showed the best performance compared to the other substrates, in terms of growth parameters such as spawn, mycelium running rate and time required for primordia initiation. The decomposed palm bunch conclusively produced the highest number of pin-head while decomposed saw dust had no pin-head. The experimental observation revealed that pin-head germinated 4 days on decomposed palm bunch without delay. Okhunaya and Okigbo (2009) had earlier reported that oil palm bunch fibres were good substrates for *Pleurotus tubergium* and other mushrooms. The performance of decomposed palm bunch might be attributed to its high amount of K and P components which could be responsible for the increase in the soil pH and subsequently enhanced quick absorption of nutrients that are essential for good growth and development of mushroom (Ojeniyi *et al*., 1998). The ineffectiveness of the decomposed sawdust as substrates for the mushroom development could be attributed to the fact that they were not properly allowed to decompose through partial composting before use which led to lowering their C/N ratio and delaying the availability and release of its nutrients for mushroom uptake and development. This result aligned to the report of Adebayo and Olayinka (1984) that used unprocessed sawdust to grow maize and found out that its high C/N ratio 1:135 made it difficult for quick release of nutrients.

Therefore, decomposed Palm bunch would act as a very suitable medium in the production of edible wild mushroom in Rivers State while decomposed Saw dust should be subjected to further research work to ascertain the problem encountered with it. Also works are ongoing to sustain the growth of the pin-head (primodia) into fruiting bodies to kick-start the artificial cultivation and production of *Agaricus silvaticus* in Rivers State, Nigeria.

CONCLUSION

The domestication of edible wild mushroom *Agaricus silvaticus* was experimentally determined to be possible in Rivers State, Nigeria if precautionary measures are put in place and due diligence followed in the process of production. This has been established from all parameters studied. Considering the successful mycelium production proved in the various soil samples collected from different sites. This research work has also given us insight into the various stages of *Agaricus silvaticus* domestication namely; Mycelium, spawn, pin-head and fruiting bodies. Spawn production

was also successful because of the impressive yield in mycelium after 7days of closed observation and monitoring. This was so as mycelium colonized spent grain after 9days of spawning which signify a huge success in growth of mushroom (*Agaricus silvaticus*). It is also worthy of note to state that of all the substrates used, palm bunch proved to be more effective substrates in course of this research.

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